RESEARCH REPORT

NEMATODE RESISTANCE SCREENING IN *MUSA* SPP. USING MACROPROPAGATED PLANTLETS

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ABSTRACT

Olajide, E., L. Cortada, K. Saikai, D. Amah, D. Coyne, W. Bert, R. Swennen, and Y. A. Kolombia. 2025. Nematode resistance screening in *Musa* spp. using macropropagated plantlets. Nematropica 55:18-31.

This study evaluated macropropagated plantlets for nematode resistance screening in *Musa* spp. We aimed to determine the optimal timing for assessing resistance to *Radopholus similis* and *Meloidogyne arenaria* in the plantain cultivar 'Agbagba' at 30-, 60-, and 90-days post-inoculation (DPI). Additionally, we tested different substrates for early-stage screening at 7 DPI. Results indicated that 60 DPI is optimal for resistance screening against *R. similis* due to significant root damage, while *M. arenaria* reproduction peaked at 90 DPI without extensive root deterioration. The sand-absorbent polymer (SAP) substrate was most effective for early nematode penetration. Using these findings, we assessed five *Musa* genotypes at 60 DPI; 'SH 3142' and 'Pisang Jari Buaya' showed resistance to *R. similis*, 'Agbagba' was susceptible, 'Yangambi KM5' was unexpectedly susceptible, and 'Pisang Lilin' showed intermediate resistance. This study highlights that macropropagated plantlets can be used as an effective method for rapid preliminary resistance screening in *Musa* spp.

Key words: Burrowing nematode, *Meloidogyne arenaria*, nematode reproduction, phenotyping, plantain, *Radopholus similis*, susceptibility

RESUMEN

Olajide, E., L. Cortada, K. Saikai, D. Amah, D. Coyne, W. Bert, y Y. A. Kolombia. 2025. Cribado de resistencia a nematodos en *Musa* spp. utilizando plántulas macropropagadas. Nematropica 55:18-31.

Este estudio evaluó el uso de plántulas macropropagadas para el cribado de resistencia a nematodos en *Musa* spp. Nuestro objetivo fue determinar el momento óptimo para evaluar la resistencia a *Radopholus similis* y *Meloidogyne arenaria* en el cultivar de plátano 'Agbagba' (tipo Hartón) a los 30, 60 y 90 días después de la inoculación (DPI). Además, probamos diferentes sustratos para el cribado en etapas tempranas a los 7 DPI. Los resultados indicaron que 60 DPI es óptimo para el cribado de resistencia contra *R. similis* debido al daño significativo en las raíces, mientras que la reproducción de *M. arenaria* alcanzó su pico a los 90 DPI sin deterioro extenso del sistema radicular. El sustrato de arena y polímero absorbente (SAP)

fue el más efectivo para la penetración temprana de nematodos. Utilizando estos hallazgos, evaluamos cinco genotipos de Musa a los 60 DPI; 'SH 3142' y 'Pisang Jari Buaya' mostraron resistencia a *R. similis*, 'Agbagba' fue susceptible, 'Yangambi KM5' resultó inesperadamente susceptible, y 'Pisang Lilin' mostró resistencia intermedia. Este estudio resalta la macropropagación como un método efectivo para el cribado rápido preliminar de resistencia en *Musa* spp.

Palabras clave: Fenotipado, *Meloidogyne arenaria*, nematodo barrenador, plátano, *Radopholus similis*, reproducción de nematodos, susceptibilidad

INTRODUCTION

Plantains and bananas (*Musa* spp.), crucial crops for food security, face a significant threat from plant-parasitic nematodes (PPNs). These pests threaten crop yield and, as a result, impact global food supply chains (Coyne, 2021; Price, 1995; Sikora et al., 2018). Nematode-infected planting materials commonly result in poor yields due to root damage inflicted by PPNs, notably the burrowing nematode (*Radopholus similis*) and the root-knot nematode (*Meloidogyne* spp.). Such damage compromises host plant access to and uptake of available water and nutrients, resulting in reduced growth and yield. Nematode damage to the root system also affects plant anchorage and consequent susceptibility to being uprooted during strong winds. The resulting agricultural losses have major economic implications, leading to billions of $US in lost revenue annually with profound effects on farmers' livelihoods and developing nations' economies (Bernard et al., 2017).

To manage the economically important PPNs on *Musa*, more innovative crop protection options are needed. Cultural management practices, such as crop rotation, cover crops, and mulching, have limited success in reducing nematode densities and improving soil health, while the cost and environmental impact of nematicides further complicate their use (Talwana et al., 2016; Viaene et al., 2003). The use of host-plant resistance against nematodes offers a safe, sustainable, and environmentally friendly solution; thus, exploring genetic resistance within *Musa* genotypes is a promising, underutilized strategy. The challenge lies in accurately identifying and deploying *Musa* genotypes inherently resistant to PPNs. Due to numerous constraints, phenotyping for PPN resistance is currently not fully developed or applied in these pest control strategies.

The current limitations in phenotyping for PPN resistance can be attributed to several factors: the complexity of assessing nematode-related traits, labor-intensive techniques, lack of robust molecular markers, genotype-specific responses, environmental variability, and the need to integrate omics data (Coyne et al., 2009; Coyne et al., 2010; Coyne et al., 2018; Kamira et al., 2013). The research presented here focuses primarily on bridging the gap in the quest for a reliable, high-throughput mechanism for the early detection of nematode-resistant *Musa* genotypes. *Radopholus similis* is particularly problematic due to its ability to overcome genetic resistance, a complex interplay between plant genotypes and pathogen biology. Research in Uganda showed how certain *R. similis* populations can overcome resistance against the pest and infect *Musa* genotypes previously identified as resistant (Plowright et al., 2013).

Traditional screening methods for PPN resistance in *Musa* spp. often rely on field or screenhouse trials. While informative, these methods face challenges, such as environmental variability, seasonality, and the long duration required before phenotypic expression of resistance become evident.

Field trials cannot represent the wide range of conditions under which the plants must perform, and in screenhouse settings, using sword suckers or tissue culture plants for resistance screening is laborious and difficult to scale due to space constraints and the challenge of generating large quantities of homogeneous plant material (De Schutter et al., 2001). To circumvent these limitations, our study utilized recent advancements in macropropagation techniques, as a promising, rapid, cost-effective solution for generating uniform planting material necessary for effective phenotyping of *Musa* germplasm. Macropropagation is a simple and effective technique used to rapidly produce large quantities

of planting material through controlled bud stimulation (Faturoti et al., 2002). This novel approach, which we adopt for nematode resistance screening, offers a more controlled, rapid, and scalable solution than conventional methods. Screening with potted plants, especially those derived from macropropagation, also allows for greater control over experimental conditions compared to field trials (Baiyeri & Aba, 2005, 2007; Faturoti et al., 2002). However, it is important to note that the substrate used in pot trials can significantly influence the outcome of resistance screening (Pitcher, 1975). Different substrates play a crucial role in affecting nematode mobility and reproduction and impacting root development and overall plant health (Hodge et al., 2009). One of our study objectives was to compare different substrates for their effectiveness in supporting nematode infection and plant response. This approach builds on previous screening studies focused on identifying resistance in *Musa* spp., including the works of Talwana et al. (2016) and Viaene et al. (2003).

The International Institute of Tropical Agriculture (IITA) has long been involved in the traditional breeding of bananas and plantains (Ortiz, 2003) and more recently for the specific objective of developing pest and disease resistant hybrids for Africa. This includes the identification of nematode-resistant genotypes within the *Musa* genus, crucial due to the limited number of resistant materials found in Africa. Efforts have included assessing the response of known cultivars to *R. similis*, identifying varying susceptibility among AAB subgroup genotypes, and resistance in certain AA genotypes. However, despite these advances, a comprehensive screening of diverse *Musa* germplasm, including wild relatives, landraces, and cultivated species, is yet to be conducted for nematode resistance. Hence, the need for robust and scalable screening methods is urgent, particularly for comprehensive assessments across diverse *Musa* germplasm, and for a more efficient high-throughput mechanism to screen large numbers of progeny (Coyne et al., 2013; Fogain and Gowen, 1996; Price, 1994a, 1994b; Quénéhervé et al., 2009; Sarah et al., 1992; Wehunt et al., 1978).

Therefore, in light of these challenges and opportunities, this study aimed to: (i) identify the best evaluation time to assess host resistance against nematodes, using *R. similis* and *M. arenaria* on the popular nematode-susceptible cultivar 'Agbagba', at key post-inoculation intervals of 30, 60, and 90 days post-inoculation (DPI); (ii) assess the efficiency of three different substrates -- SAP substrate, steam-sterilized topsoil, and a cocopeat mix (1:1 v/v) -- for early-stage nematode screening at 7 DPI; and (iii) evaluate the response of five *Musa* reference genotypes to *R. similis* at 60 DPI. By fulfilling these objectives, we aim to standardize the use of macropropagated plantlets to phenotype *Musa* germplasm for resistance to *R. similis*, contributing to the body of knowledge on PPN management in *Musa* crops, thus offering tangible solutions to the agricultural sector.

MATERIALS AND METHODS

Plant material selection and propagation

All experimental procedures were performed using macropropagated plantlets. Sword suckers of each genotype were obtained from the International Institute of Tropical Agriculture (IITA) plantain breeding collection at Ibadan and Onne stations, Nigeria. After washing and paring to remove roots and necrotic tissues, the corms were subjected to a boiling water treatment for 30 s to eliminate pathogens (Coyne et al., 2010). The corms were then put aside for 24 hr, after which incisions were made on the apical meristems before placement in the macropropagation chamber, which provides a warm, humid environment with 50% shade to promote bud break and initial shoot development (Baiyeri and Aba, 2007; Faturoti et al., 2002). After 3-5 wk, plantlets with three well-developed leaves were extracted from the corms, transplanted into 2-L plastic nursery bags containing specific substrates, and then weaned and acclimatized for 2 wk.

For our experiments: (i) plantlets of 'Agbagba' were transplanted into steam-sterilized topsoil to determine the optimal evaluation time for host resistance to *R. similis* and *M. arenaria*. (ii) plantlets of 'Agbagba' were transplanted into three different substrates – SAP substrate, steam-sterilized topsoil, and a 1:1 v/v mixture of cocopeat and topsoil - to assess substrate efficiency for early-

stage nematode screening. (iii) plantlets of five *Musa* genotypes (Table 1) were grown in the SAP substrate, selected under Objective (ii) as the most conducive for nematode reproduction and consistent root development.

Preparation of nematode inoculum

The *R. similis* culture used in all experiments was initially isolated from plantain 'Agbagba' at IITA-Ibadan, Nigeria. Cultures were maintained on carrot discs according to Marin et al. (2000). Nematodes were extracted using the modified Baermann tray technique (Coyne et al., 2014).

A population of *M. arenaria*, originally isolated from plantain 'Agbagba' in Nigeria (Olajide et al., 2023) and identified using NAD5 mtDNA techniques (Janssen et al., 2016), was used in this study. The *M. arenaria* were maintained on the susceptible tomato 'Marmande' in a screenhouse. Nematode eggs were extracted from heavily galled roots using the sodium hypochlorite method (Hussey and Barker, 1973). Second-stage juveniles (J2s) were obtained by hatching eggs on a modified Baermann extraction tray for 3-7 days. For inoculation, J2s of *M. arenaria* and mixed life stages (excluding eggs) of *R. similis* were concentrated by gently removing excess water. The nematodes in the suspensions were counted, and density calculated from three aliquots under a microscope. Inoculation densities were standardized based on substrate volume following established protocols (Marin et al., 2000). Specifically, 1,000 nematodes were applied to 2 L of substrate, and 200 nematodes to 0.4 L to ensure consistency across experimental setups. A volume of 1 mL containing the desired nematode population was inoculated into three, 1-cm-deep holes around the stem of each plant, following a method adapted from Coyne et al. (2014). Control plants were inoculated with sterile water. The experiments were conducted in a IITA-Ibadan screenhouse facility during 2019–2021 (7°22′39″N, 3°54′21″E).

Optimal evaluation time for host resistance in 'Agbagba'

After 3-5 weeks of development in the chambers, macropropagated plantlets were transplanted into 2-L plastic nursery bags filled with steam-sterilized topsoil and then weaned and acclimatized for 2 wk. Following acclimatization, the plantlets were inoculated with 1,000 mixed life-stages (no eggs) of *R. similis*, while another set of plantlets was inoculated with 1,000 J2s of *M. arenaria*. Plants were assessed for root weight and nematode population density at 30, 60, and 90 DPI. The DPI intervals were selected to coincide with key stages in the nematode life cycle and the plant's defense response, with 30 DPI representing early interactions, 60 DPI aligning with peak nematode reproduction, and 90 DPI capturing the potential cumulative effects of infection. The controls were not inoculated with nematodes, and plants were watered every 2-3 days to maintain consistent

Table 1. Characteristics of five reference *Musa* genotypes used in experiments.

Genotype	Genome	Type	Reaction to *R. similis*	Source(s)
Agbagba	AAB	Plantain	Susceptible	Coyne et al., 2013
Pisang Lilin	AA	Banana	Resistant	Seenivasan, 2017
Yangambi KM5	AAA	Banana	Resistant	Fallas & Marbán-Mendoza, 1994; Fogain & Gowen, 1998; Hahn et al., 1996; Pinochet, 1979; Price, 1994b
Pisang Jari Buaya	AA	Banana	Resistant	Pinochet, 1979; Price, 1994a, 1994b; Udomkun et al., 2021;Wehunt et al., 1978
SH 3142	AA	Banana	Resistant	Viaene et al., 2003

moisture levels. At each DPI, *M. arenaria* were extracted using the Hussey & Barker (1973) NaOCl method, while *R. similis* were extracted using the modified Baermann tray technique (Coyne et al., 2014). The final nematode population density (Pf) per plant was estimated from a 10 g sub-sample of fresh root. For each DPI (30, 60, and 90 days), root fresh weights were recorded for both inoculated and corresponding non-inoculated control plants in each 2-L plastic nursery bag. The experiments were conducted using a completely randomized design (CRD), with each treatment replicated five times. The entire experiment was conducted twice independently under the same conditions.

Differences in root weights between inoculated and non-inoculated plants at 30, 60, and 90 DPI were analyzed using Student's *t*-test. Nematode population densities were assessed using one-way ANOVA followed by Tukey's post-hoc test.

Substrate selection for nematode screening

SAP substrate (Reversat et al., 1999), topsoil, and a mixture of cocopeat (50%): topsoil (50%) [1:1 v/v ratio] were selected based on the results of preliminary experiments (data not shown). The topsoil used, classified as loamy soil, was sourced from virgin forest-derived materials in Ibadan, Nigeria. The cocopeat was also sourced in-house by IITA to ensure quality and consistency. Both the topsoil and cocopeat were steam sterilized at 121°C for 20 min before use.

The effectiveness of SAP in screening rice germplasm has been documented (Reversat et al., 1999). To prepare the SAP substrate, 37.5 g polymer (DCM Aquaperla®) was added to 3,750 mL distilled water, allowing the mixture to absorb fully over 2 hr. After absorption, the swollen polymer was blended in 500-mL batches for 3 cycles of 20 sec, with 5-s intervals between cycles. Autoclaved river sand (sterilized at 121°C for 20 min) was thoroughly mixed with the polymer gel to achieve a homogeneous mixture. This prepared substrate was spread out in a thin layer and left to dry under cover, protected from direct sunlight, for 72 hr, resulting in a dry, sandy, granular consistency.

At 3-5 wk, plantlets with three well-developed leaves were extracted from the corms and transplanted into 0.4-L plastic cups, each filled with the substrate, where they were weaned and acclimatized for 2 wk. They were then inoculated with approximately 200 mixed life stages (no eggs) of *R. similis*. Nematode penetration of roots was compared among the three selected substrates: sand and SAP substrate, topsoil, and a 1:1 v/v mixture of cocopeat and topsoil at 7 DPI. After the incubation period, plants were removed, and the whole root system was gently washed with tap water, then stained using acid fuchsin to visualize the nematodes (Bridge & Page, 1980). The total number of nematodes inside each root system was then counted under a stereomicroscope. The penetration study was conducted twice with five replicates per treatment. One-way ANOVA was performed separately for nematode penetration, shoot length, root length, and root weight across the three substrates, followed by Tukey's post-hoc test to identify significant differences.

Assessing Musa genotype responses to Radopholus similis

Building on the results from our substrate comparison and timing assessments, we utilized the SAP substrate, which effectively supported nematode penetration, and a 60 DPI timeframe, identified as optimal for nematode reproduction observation. The plantlets of the genotypes 'Yangambi KM5', 'Pisang Lilin', 'SH 3142', 'Pisang Jari Buaya', included as resistant references, and 'Agbagba' as a susceptible control (Table 1), were produced using the macropropagation technique to validate the consistency of macropropagated plantlets in resistance screening. Macropropagated plantlets acclimatized in 0.4-L SAP substrate were inoculated with 200 mixed life-stages (no eggs) of *R. similis*. The experiment was terminated at 60 DPI and was repeated once with five replications per treatment.

To assess the reproduction of *R. similis*, a 10 g root sub-sample was collected from each plant after all roots had been chopped and thoroughly mixed, and nematodes were extracted using the modified Baermann tray technique (Coyne et al., 2014). The Pf per plant, defined as the total number of infective nematodes per root system, was calculated, and the reproduction factor (RF = Pf/Pi) determined. On five randomly selected functional

Musa roots, the percentage root necrosis was assessed following Speijer et al. (1997). The selected roots were cut to ~5 cm in length to accommodate the smaller size of roots available in our study. Despite this modification, each root segment still contributed a maximum score of 20%, with the total score for the five segments reaching 100%. Then, each root segment was sliced lengthwise, and percentage necrosis was rated.

The Pf, RF, and percentage root necrosis among genotypes were compared using one-way ANOVA, followed by Tukey's post-hoc test for significant differences.

Statistical analysis and data interpretation

All statistical analyses were conducted using R statistical software, version 3.6.2 (R Core Team, Vienna, Austria). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. Log-transformation ($\log_{10}[x + 1]$) was applied when necessary. Repeated experiments were pooled after confirming no significant interactions ($P > 0.05$), with significance set at $P \leq 0.05$.

RESULTS

Optimal timing for host resistance evaluation of Musa to Radopholus similis and Meloidogyne arenaria

At 60 DPI, *R. similis*-infected plants exhibited dark brown to black lesions on the root cortex, root necrosis, and extensive root decay (Fig. 1B). In contrast, *M. arenaria*-infected plants developed small galls on the roots after 90 DPI without significant root decay (Fig. 1A). For *R. similis*, at 30 DPI, no significant difference was observed in root weight between the non-inoculated and inoculated plants ($P = 0.487$; Fig. 2A). However, at 60 and 90 DPI, the root weight of *R. similis*-inoculated plants was significantly lower than that of the non-inoculated plants ($P < 0.001$; Fig. 2A). The root weight of non-inoculated plants increased from 30 to 90 DPI, while in inoculated plants, root weight consistently decreased over time. Furthermore, the total number of *R. similis* was significantly higher at 60 DPI compared to 30 and 90 DPI ($P< 0.001$; Fig. 2B). These results indicate that screening with macropropagated plantlets for *R. similis* is most efficient at 60 DPI.

For *M. arenaria*, at all time points (30, 60, and 90 DPI), no significant differences were observed in root weight between inoculated and non-inoculated plants ($P > 0.05$ at all time points), and root weight increased over time for both groups (Fig. 3A). The total number of *M. arenaria* increased from 30 to 90 DPI ($P < 0.001$; Fig. 3B).

Substrate efficacy in nematode screening

There were no differences among the three substrates for plantain 'Agbagba' concerning shoot length, root length, and root weight ($P > 0.05$).

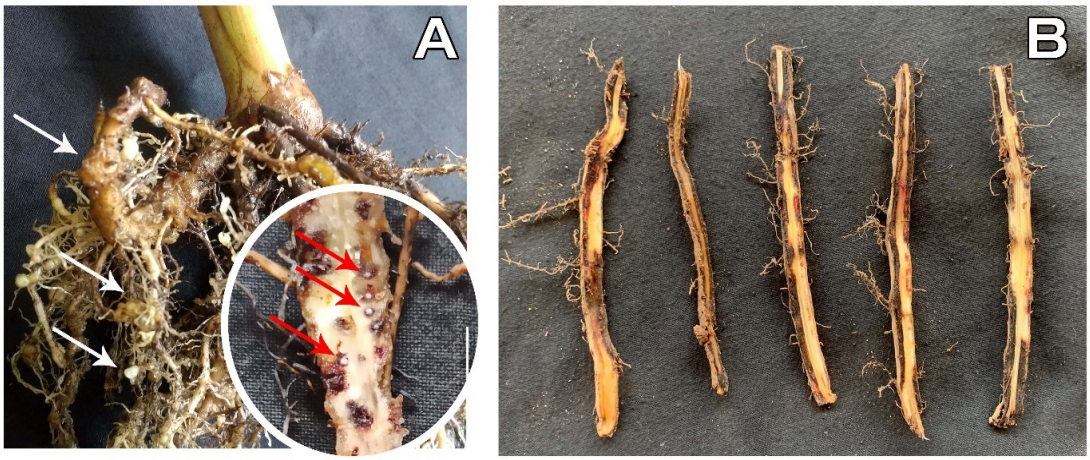

Figure 1. Plantain 'Agbagba' infected roots with (A) galling (white arrows) and females (red arrows) of *Meloidogyne arenaria* infection at 90 days post inoculation (DPI). (B) Necrotic lesion caused by *Radopholus similis* at 60 DPI.

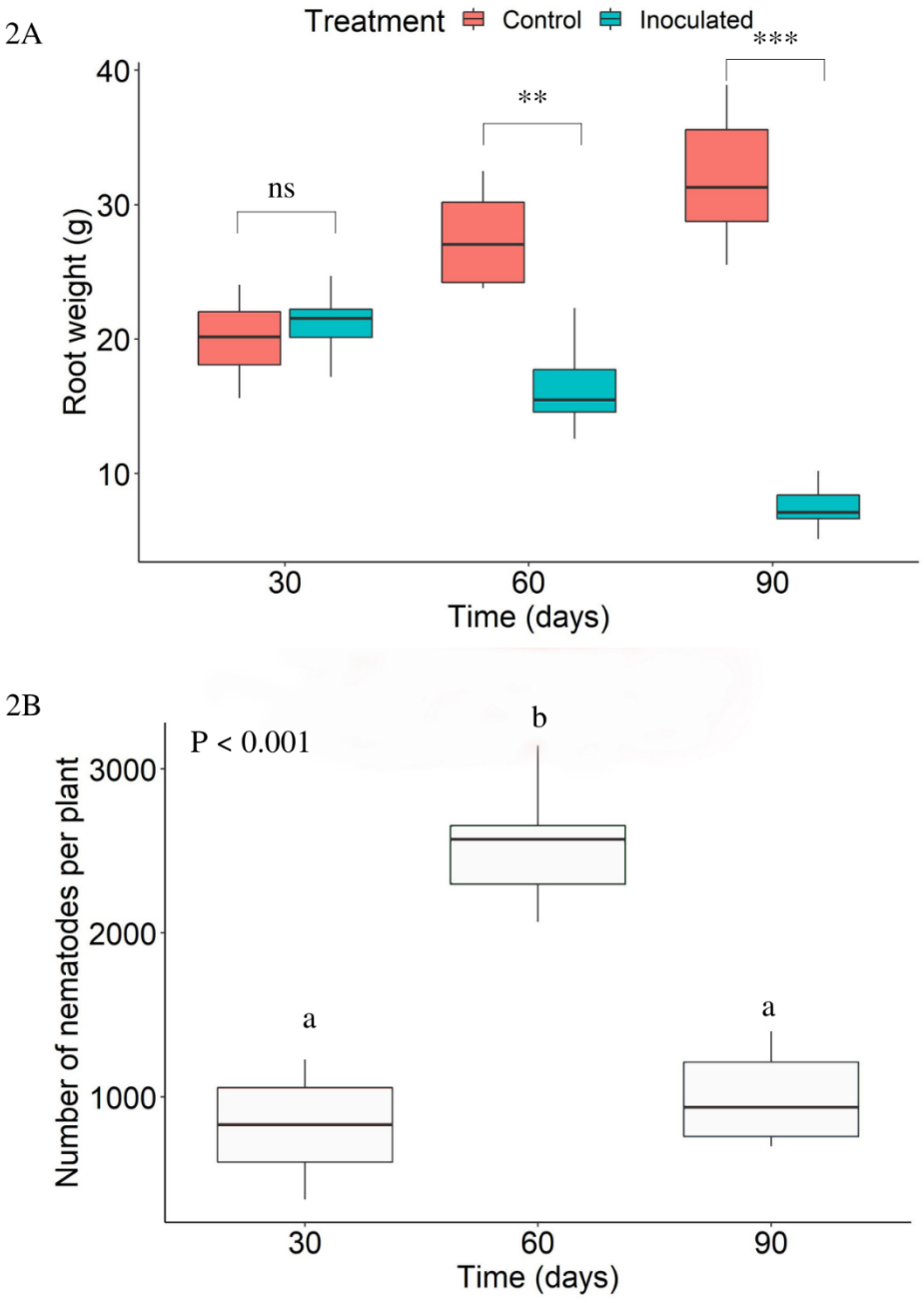

Figure 2. Root weight and reproduction of *Radopholus similis* at 30-, 60- and 90-days post inoculation (DPI) on plantain 'Agbagba'. (2A) Fresh root weight (g) between non-inoculated and inoculated plantlets at 30, 60 and 90 DPI (ns, not significant), **: *P*-value <0.01, ***: *P*-value <0.001. (2B) Final *R. similis* population density in roots at 30, 60 and 90 DPI. Data represent means ± SE (N = 10). The same letter on the boxplot are not significantly different ($P \leq 0.05$).

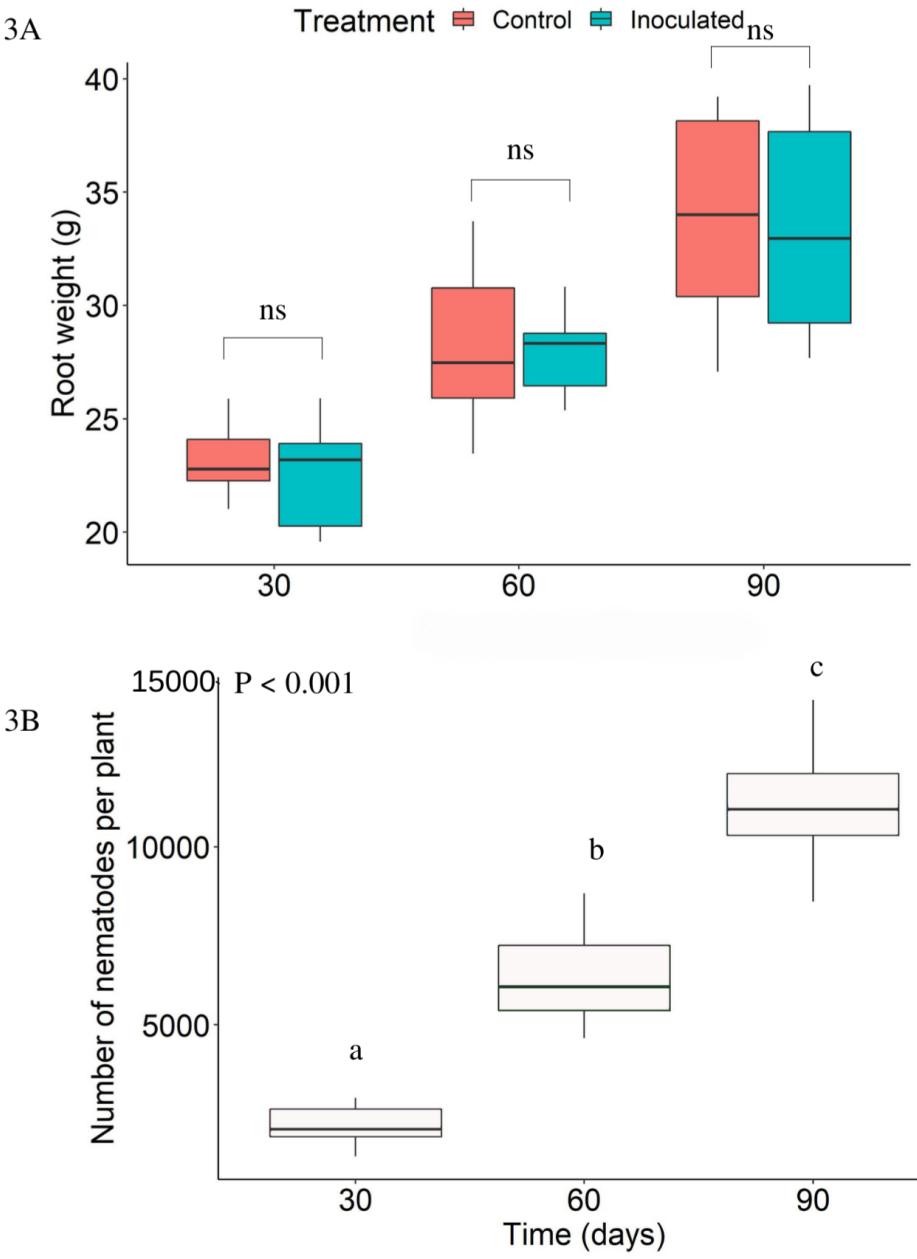

Fig. 3. Root weight and reproduction of *Meloidogyne arenaria* at 30, 60 and 90 days post inoculation (DPI) on cv. 'Agbagba'. (A) Fresh root weight (g) of non-inoculated and inoculated plantlets at 30, 60 and 90 DPI (ns, not significant). (B) Final *M. arenaria* population density in roots at 30, 60 and 90 DPI. Data represent means ± SE (N = 10). The same letter on the boxplot are not significantly different ($P \leq 0.05$).

However, *R. similis* penetration of roots in SAP was significantly higher ($P < 0.01$) compared to nematode penetration of roots in topsoil and the 1:1 v/v mixture of cocopeat and topsoil (Table 2).

Resistance screening of reference Musa genotypes

No significant differences in shoot length, root length, and root weight among genotypes were found ($P > 0.05$; Table 2; Table S1). However, the *R. similis* Pf and RF were significantly higher in plantain 'Agbagba' and 'Yangambi KM5'; compared to 'SH 3142' and 'Pisang Jari Buaya' at both 30 and 60 DPI ($P < 0.001$). There were no significant differences in percentage root necrosis measured at 60 DPI among genotypes ($P = 0.419$; Table 3).

The data indicate differences among genotypes in host suitability for *R. similis*. 'SH 3142' and 'Pisang Jari Buaya' had significantly lower Pf and RF values ($P < 0.05$), indicating possible resistance, compared with 'Agbagba' and 'Yangambi KM5', which were susceptible. 'Pisang Lilin' showed an intermediate response, as the Pf and RF values were between those of the resistant and susceptible genotypes, although the differences were not statistically significant ($P > 0.05$).

DISCUSSION

Our study effectively pinpointed the optimal timing for resistance screening, identified a suitable substrate for early-stage *R. similis* penetration, and determined the host response of five reference *Musa* genotypes. We demonstrated that the optimal timing for resistance screening for *R. similis* was at 60 DPI. At 60 DPI, the roots had suffered severe damage from *R. similis*, leading to the root system being unable to sustain itself. In contrast, *M. arenaria* reached the highest reproduction at 90 DPI without entirely destroying the roots. Previous studies using tissue culture plant material have reported an increase in *M. incognita* and *M. javanica* Pf, which could be attributed to a slightly extended exposure time (Pinochet et al., 1998). Future studies should explore additional resistant and susceptible *Musa* genotypes and confirm the optimal screening timing under varied environmental conditions to strengthen and expand upon these findings.

Table 2. *Radopholus similis* penetration, shoot length (cm), root length (cm), and root weight (g) on plantain 'Agbagba' grown in three substrates: SAP substrate, topsoil, and cocopeat:topsoil at 7 days post inoculation (DPI) using macropropagated plantlets (N = 10).

Substrate	Nematode penetration (number of nematodes per root system)	Shoot length (cm)	Root length (cm)	Root weight (g)
SAP	28.2 ± 1.9 b[z]	14.8 ± 0.6 a	14.1 ± 0.6 a	16.3 ± 0.5 a
Topsoil	10.1 ± 1.8 a	13.3 ± 0.7 a	14.4 ± 0.6 a	15.1 ± 0.7 a
Cocopeat:topsoil	13.1 ± 1.4 a	13.9 ± 0.7 a	13.4 ± 0.5 a	15.8 ± 0.7 a

[z]Data represent means ± SE (N = 10). Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Different letters in the same column indicate means that were statistically different ($P \leq 0.05$).

Table 3. *Radopholus similis* reproduction on *Musa* genotypes at 60 days post inoculation (DPI).

	60 DPI		
Genotype	Number of nematodes (Pf)[x]	Reproduction factor (RF) values[y]	% Root necrosis
Agbagba	4,058 ± 579 a[z]	20.3 ± 2.9 a	19.2 ± 1.7 a
Pisang Jari Buaya	622 ± 114 b	3.1 ± 0.6 b	15.1 ± 1.1 a
Pisang lilin	2,142 ± 405 ab	10.7 ± 2 ab	17.7 ± 1.8 a
SH 3142	1,218 ± 268 b	6.1 ± 1.3 b	15.8 ± 1.4 a
Yangambi KM5	3,857 ± 632 a	19.3 ± 3.2 a	17.1 ± 2 a

[x]Pf = final nematode population density.
[y]RF = Pf/Pi [initial nematode population density (200 mixed stages *R. similis*)].
[z]Data for each time-point were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Data represent means ± SE (N = 10).

The selection of a substrate is critical for PPN screening and requires a substrate that facilitates nematode penetration and reproduction, while ensuring consistent root and shoot growth. The SAP substrate facilitated significantly higher nematode penetration at 7 DPI compared to topsoil and the cocopeat-topsoil mixture. This aligns with previous studies indicating that sandy substrates enhance nematode movement due to better aeration and moisture availability (Marin et al., 2000). Therefore, SAP substrate is recommended for standardizing nematode resistance assays in *Musa* spp.

Numerous studies have investigated the *Musa* plant resistance against various PPNs, particularly *R. similis*, with several genotypes reported to have good resistance against *R. similis* (Coyne et al., 2013; Pinochet, 1979; Viaene et al., 2003; Wehunt et al., 1978), and these materials are often included in screening studies as reference controls. Our findings indicated that 'Pisang Jari Buaya' and 'SH 3142' are resistant, confirming earlier reports (Pinochet, 1979; Price, 1994a, 1994b; Udomkun et al., 2021; Viaene et al., 2003; Wehunt et al., 1978). However, we could not confirm the resistance status of 'Pisang Lilin' and 'Yangambi KM5' as the reproduction of *R. similis* was similar to that of the susceptible control 'Agbagba'. This variation in host response may be attributed to intraspecific differences in the pathogenicity of *R. similis* (Fallas and Marban-Mendoza, 1994; Fogain and Gowen, 1998; Hahn e*t al*., 1996; Pinochet, 1979; Price, 1994b; Seenivasan, 2017). These findings suggest that the resistance of 'Pisang Lilin' and 'Yangambi KM5' may depend on the specific *R. similis* population encountered. Future studies should investigate the resistance of these *Musa* genotypes against diverse *R. similis* populations. This remains one of the many challenges plant pathologists and *Musa* breeders are facing with screening for *Musa* resistance to PPNs.

Variability in the pathogenicity and virulence of *R. similis* populations is well-known (Plowright et al., 2013). For example, Plowright et al. (2013) reported differences in the aggressiveness of Ugandan *R. similis* populations on two widely recognized resistant genotypes 'Pisang Jari Buaya' and 'Yangambi KM5', indicating that resistance may be pathotype-specific. However, in West Africa and other regions, there is limited information on the different levels of aggressiveness among isolates of *R. similis* and other PPNs. Understanding variations in pathogenicity and virulence patterns of PPNs across all *Musa*-growing regions could significantly benefit *Musa* breeding programs. It would help minimize variability in screening results across sites and locations and facilitate the identification and deployment of functional resistance genes for gene pyramiding.

The consistent genotype responses observed in this study, particularly the resistance of 'SH 3142' and 'Pisang Jari Buaya' and the susceptibility of 'Agbagba' to *R. similis*, confirm that macropropagated plantlets are effective for assessing the host response of banana and plantain genotypes to nematodes. This highlights the utility of macropropagation as a simple, practical, and reliable screening method for resistance evaluation in *Musa* breeding programs. Due to its simplicity, cost-effectiveness, and ability to produce consistent, nematode-free planting materials, macropropagation is recommended as a standard approach for resistance screening, especially in resource-constrained settings where other methods may not be feasible. The findings from this study contribute valuable insights for optimizing nematode resistance screening in *Musa* spp., aiding in the identification and development of resistant cultivars and inform integrated pest management strategies.

While macropropagation offers a promising alternative to traditional propagation methods, it is imperative to acknowledge observed variability in propagation efficiency among different *Musa* genotypes. This variability mirrors the challenges encountered in tissue culture propagation, where certain genotypes exhibit more robust proliferation than others. Addressing this variability will be crucial for ensuring the widespread adoption and success of macropropagation in *Musa* breeding programs. Researchers should explore the underlying genetic and physiological factors that contribute to differential propagation efficiency, potentially through comparative transcriptomic or metabolomic analyses. By identifying the key molecular markers associated with enhanced propagation, targeted breeding efforts can be undertaken to develop *Musa* genotypes that are more amenable to macropropagation. Additionally, optimizing the macropropagation protocols, such as adjusting the media composition or

environmental conditions, may help mitigate the impact of genotypic variability and further improve the consistency of results.

In conclusion, our study highlights the effectiveness of using macropropagated plantlets for nematode resistance screening in *Musa* spp. By identifying 60 DPI as the optimal evaluation time for *R. similis* and demonstrating the suitability of the SAP substrate for nematode penetration, we provide a framework that can expedite and standardize resistance screening processes in *Musa* spp. These advancements are valuable for breeding programs aiming to develop nematode-resistant *Musa* cultivars, especially in resource-limited settings.

ACKNOWLEDGMENTS

This work was supported by the United States Agency for International Development (USAID), Special Research Funds Ghent University (BOF18/DOS/066), the Breeding Better Banana (BBB) project, and the International Institute of Tropical Agriculture (IITA).

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Received: *25/IX/2024*

Accepted for publication: *5/II/2025*

Recibido:

Aceptado para publicación:

SUPPLEMENTARY DATA

Table S1. Shoot length, root length, and root weight of *Musa* genotypes at 7, 30, and 60 days post inoculation (DPI) with *Radopholus similis*.

Genotypes	7 DPI			30 DPI			60 DPI		
	Shoot length (cm)	Root Length (cm)	Root weight (g)	Shoot length (cm)	Root Length (cm)	Root weight (g)	Shoot length (cm)	Root Length (cm)	Root weight (g)
Agbagba	12.2 ± 0.6	11.1 ± 0.5	11.8 ± 1.5	14.2 ± 1.2	18.4 ± 1.3	18.1 ± 1	16.4 ± 0.6	21.7 ± 1.2	30.0 ± 3
Pisang Jari Buaya	12.2 ± 0.6	11.1 ± 0.5	14.5 ± 2.1	13.5 ± 0.9	20.2 ± 0.9	20.8 ± 1.5	15.9 ± 0.5	19.4 ± 1.1	31.9 ± 2.2
Pisang lilin	12.1 ± 0.6	12.0 ± 0.5	12.4 ± 1.8	12.6 ± 0.9	18.9 ± 1	20.1 ± 1.3	16.0 ± 0.6	19.5 ± 0.8	23.6 ± 2
SH 3142	12.3 ± 0.7	11.2 ± 0.5	10.7 ± 1.9	13.2 ± 0.6	19.4 ± 1.3	17.7 ± 1.3	16.0 ± 0.6	19.9 ± 1.6	46.3 ± 4.1
Yangambi KM5	12.0 ± 0.6	11.3 ± 0.3	12.4 ± 1.1	13.8 ± 0.7	19.3 ± 1.2	19.6 ± 1.7	16.9 ± 0.7	21.6 ± 1.3	31.0 ± 3.5
	ns	ns	Ns	ns	ns	ns	ns	ns	ns

ns: not significantly different ($P \leq 0.05$). Data represent means ± SE (N = 10). Data for each time-point for shoot length, root length, and root weight were analyzed by one-way ANOVA followed by Tukey's post-hoc test.